



# Biopharmaceutical Emerging Best Practices Association

## Statistics for Validating Potency Assays

### *BEBPA Technical Note*

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There has been much churning and upgrading of potency assays validations. The “old school” follows ICH Q2 (R1) entitled; **Validation of Analytical Procedures: Text and Methodology Q2 (R1)**. This guideline published by the International Conference on Harmonisation (ICH) was first released 27 October 1994 and incorporated in its current format in November 2005. It is available electronically at:

[https://www.ich.org/fileadmin/Public\\_Web\\_Site/ICH\\_Products/Guidelines/Quality/Q2\\_R1/Step4/Q2\\_R1\\_Guideline.pdf](https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1_Guideline.pdf).

In the 1990s, this document was a breakthrough document as it finally provided a common set of terms and proposed assay characteristics which every development lab around the world could use for all types of analytical methods. This is the document which your Quality Assurance group will likely use to determine if your proposed bioassay method validation is acceptable. It is my strong suggestion, that whenever possible you utilize the terminology proposed in this guideline. The key terms identified and defined in this document are:

1. Types of methods:
  - a. Identification tests
  - b. Quantitative tests for impurities' content
  - c. Limit tests for control of impurities
  - d. Quantitative tests of the active moiety
2. Method characteristics
  - a. Accuracy
  - b. Precision: Repeatability (within assay) and Intermediate (between assay).
  - c. Specificity
  - d. Detection Limit
  - e. Quantitation Limit
  - f. Linearity
  - g. Range

The potency bioassay is considered to be a quantitative test of the active moiety. As such, ICH Q2 (R1) identifies the following characteristics be included in your validation protocol: Accuracy, Repeatability, Intermediate Precision, Specificity, Linearity and Range.

The problem raises its ugly head when we consider the reportable value. Unlike most analytical methods, the potency often requires multiple runs of an assay, which are then combined to yield a reportable value. When Q2 was being written, this was not a consideration. Most analytical methods require one or at most 2 assay runs and can be completed in a single workday. However, potency assays typically require 3 or more assay runs and often require several weeks to complete. Therefore, to perform a “classic” Q2 style validation would mean running each sample 3 to 5 times, and three plates per sample. This can sometimes take upwards of a year to complete.



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Addressing this problem, the USP published <1033> Biological Assay Validation (available electronically at [https://www.ipqpubs.com/wp-content/uploads/2010/06/USP\\_1032.pdf](https://www.ipqpubs.com/wp-content/uploads/2010/06/USP_1032.pdf)). One of the more practical (and radical) concepts in this chapter is that one does not need to run the method exactly the number of times required by the assay SOP in order to assess the accuracy and precision of the method. Instead, one determines the precision of the individual assay runs required to support the proposed specification and validates this value. This approach often requires 1/3 the number of runs, but also requires the following statistical tools:

1. Utilization of assay capability to determine the required assay run precision
2. Method to combine precision numbers from various concentrations and various runs to estimate the assay run precision.

So, the “new” statistical tools you will need are:

- Power calculations to determine the number of runs needed within the validation
- Assay capability calculation to help establish the validation criteria
- Variance Component Estimates to estimate the precision of the assay runs
- A procedure on how to combine these variance estimates to minimize the number of runs needed for the validation.

Sadly, this is a bit above the pay grade of most bench level bioassay analysts who are taxed with designing and executing the potency validation. My suggestions?

1. Utilize the example within the USP, put the numbers into your preferred statistical software (even excel will work) and perform the calculations. This will ensure your calculation approach meets the recommendations within the USP.
2. Enlist the help of a statistician to design the validation. However, make sure that you use the terminology from ICH Q2, otherwise you will not pass your internal QA group.
3. Realize that one of the most controversial aspects of the chapter <1033> is the idea that you can design the final assay format after the validation. I personally have taken the middle road and pre-calculate the required assay run precision needed to support different formats. I get these values from the capability calculation. Then, I provide a table in the validation protocol which outlines to the multiple precision requirements. (See example table below):

# of runs for potency assay	1.00	2.00	3.00	4.00
Target OOS Rate (%)	0.020	0.020	0.020	0.020
Upper Specification Limit	0.70	0.70	0.70	0.70
Lower Specification Limit	1.42	1.42	1.42	1.42
<b>Target Potency Assay Precision</b>	<b>9.46%</b>	<b>13.64%</b>	<b>16.95%</b>	<b>19.81%</b>

If you want more information about the statistical tools required to do this type of validation, and believe me it is worth it – a typical ICH validation for a 3 plate format, just for precision requires 27 assay plates, while the USP-like approach requires 9 plates.